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Comparative Sero-prevalence of Herpes Simplex Virus Type-1 IgG Antibodies in Nigerian Children by Two Methods; IFA and ELISA

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Abstract

Herpes simplex virus type-1 (HSV-1) are common cause of fatal sporadic encephalitis in 70% of children and can cause chronic ulcerative infection in immuno- suppressed children leading to latency with subsequent reactivate in the conjunctiva resulting in scarring, thickening of the cornea and blindness. This comparative study determined the sero-prevalence of HSV-1 IgG antibodies in children attending some selected Hospitals in Kaduna state, Nigeria. A total of 377 blood samples were collected from children less than five years old attending some selected hospitals in Kaduna State and analyzed for HSV-1 IgG antibodies employing Enzyme immune assay technique by using commercially available ELISA Kits and Indirect Fluorescent Antibody (IFA) test. Sero-prevalence rate of 57.8 % (218 /377) was obtained by ELISA and 67.4% (255/377) by IFA test. The highest prevalence of HSV-1 infection was obtained in children in age group 49-60 months (85.2%) and lowest in children in age group 13-24 months (44.8%). Herpes simplex virus type -1 infection was significantly associated with age. Though a higher prevalence was obtained in female children than male children the difference observed in the prevalence was not statistically significant .The infection was significantly associated with children who were in school (x^2= 15.28, df = 1, P= 0.001) by only the ELISA test. Clinical symptoms significantly associated with HSV-1infection in children in this study were febrile illnesses, conjunctivitis, jaundice, skin infections and oro-facial lesions while the risk factors were age and educational status of children. Over half of the children sampled were protected from HSV-1 infection while about 40% of the children were susceptible to the infection and were at risk of developing severe HSV-1 manifestation which includes keratitis, encephalitis and Keratoconjunctivitis.

Keywords: *Comparative seroprevalence, Herpes Simplex Virus Type-1, Children, ELISA, IFA, Kaduna State, Nigeria.*

INTRODUCTION

Herpes Simplex Virus (HSV) belongs to the family *Herpesviridae* and is a large enveloped DNA virus of icosahedral symmetry divided into two types, HSV1 and HSV 2 (Vittone *et al.*, 2005; Brooks *et al.*, 2010; Willey *et al.*, 2011; WHO, 2017). The virus is an ever-present pathogen that usually causes either asymptomatic infection or skin and mucosal diseases (Fusun *et al.*, 2007) with an estimated 3.7 billion people below 50 years old infected with the infection worldwide (WHO, 2017). The major clinical manifestations associated with HSV-1 infections are gingivostomatitis, keratitis and conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and lethal infections of newborns (Fusun *et al.*, 2007; WHO, 2017). Most HSV-1 infections are acquired in childhood and the infection is lifelong with frequent or subsequent reactivations (WHO, 2017).

Neurological sequellae such as facial nerve palsy and herpes simplex encephalitis have also been associated with HSV-1 reactivation. Herpes simplex encephalitis (HSE) is the most common fetal cause of sporadic encephalitis and without treatment 70% of paediatric patients die (Kimberlin, 2004;Ibrahim *et al.*, 2005; Ward *et al.*, 2011)and accounts for 23% of serious neurological disease in children (Ward *et al.*, 2011). Neonatal herpes is a potentially devastating consequence of perinatal transmission of HSV, with significant morbidity and mortality (Leung and Sacks, 2003; Brooks *et al.*, 2010).

With the rising trend of HIV infection in the populace, more children are born every day with HIV, handling of these children by asymptomatic nurses in the health care delivery further endangers HSV infection.

The virus is not a reportable disease in Nigeria and there is a dearth of information on its seroprevalence in neonates and children. The study was therefore conducted to determine the seroprevalence of HSV-1 infection in children 0-5 years attending some selected hospitals in Kaduna state, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in Kaduna State, Nigeria. Kaduna state occupies part of the north central region of Nigeria. The Global location of Kaduna state is between 06^0 and 113^0 north of the equator and occupies an area of approximately 48,732.2 square kilometers.

Study Population

A total of 377 blood samples were randomly collected from male and female children 0-5 years old in three selected health care facilities from January 2011 to August 2011.The health care facilities include Barau Dikko Specialist Hospital (BDSH) Kaduna, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria and Institute for Child Health (ICH) Banzazzau, Zaria.

Study Design

The study was a hospital-based, cross sectional descriptive study. In all 377 children below 5 years who present with fever attending the selected hospitals during the study period were selected and enrolled in the study.

Ethical Approval

Approval for the study was obtained from the ethical committee of the hospitals (REF-ABUTH/PGO/COMMM9;BDSH/KD/SUB/322/VOL. **1)** and consents for the participation of the children in the study were obtained from parents or care givers. Prior to the sample collection, demographic and clinical information of the children were obtained using structured questionnaires administered to parents or care givers who consented to the study.

Sample Collection and Processing

Using a sterile disposable syringe, 3ml of venous blood was collected aseptically by a clinician and dispensed into a plain sterile sample bottles and transported safely to the laboratory. The blood samples were centrifuged at 2,500 rpm and serum collected into clean, sterile dry plain sample bottles using a clean dry Pasteur pipette. The sera were stored at -20^0C until needed for analysis (Cheesbrough,2000).

Analysis of Sera by Enzyme-Linked Immunosorbent Assay

The sera were tested for the presence of HVS-1 IgG antibodies using a commercially available IgG enzyme-linked Immunosorbent assay (ELISA) Kit manufactured by DIAGNOSTIC AUTOMATION, INC. USA. The ELISA uses HSV-1 antigens for the detection of anti-HSV-1 IgG antibodies in serum. The absorbance was read at 450 nm using an ELISA micro titer plate reader (Sigma Diagnostic). The presence or absence of anti-HSV-1-specific IgG antibodies in the test samples was calculated according to the manufacturer's instructions. Results were obtained by comparing the antibody titers with the cut off values of the positive and negative controls.

Analysis of Sera by Indirect Fluorescent Antibody (IFA) test

The sera was retested using IFA test, The Zeus scientific Inc, Fluorescent HSV antibody test system is designed to detect circulating HSV-1 antibodies in human sera. The system employs HSV-1 infected substrate cells coated on substrate slide and goat FITC labeled anti human immunoglobulin adjusted for optimum use (1 in 10 dilution). The reaction occurred in two steps; thefirst step is the interaction of HSV antibodies in patient's sera with the HSV infected substrate cells and Interaction of FITC labeled anti human immunoglobulin with the HSV antibodies attached to the HSV localized in the nucleus and / or cytoplasm of the infected cells. The slides were examined immediately with a fluorescent microscope at a total magnification of X250

Statistical Analysis

Data obtained were analyzed using SPSS statistical package version 17. Pearson Chi-square test of association was used to determine association between variables and seropositivity to HSV-1 infection in the children at0.05 significant levels. The seroprevalence rate to HSV-1 IgG obtained by both the ELISA and IFA test was compared using the Mann Whitney test of statistics and statistical difference (p=0.005) was found toexist between the two methods used. The measure of agreement between the two tests (ELISA and IFA) in determining the seroprevalence of HSV-1 IgG antibody was further analysed using Coshen kappa statistics and was found to be 0.534 (x^2 = 112.33, df =1, p=0.001,κ =0.534).

RESULTS

Analysis of Total Study Population

The analysis of the entire population studied showed that out of the 377 blood samples collected, 126 (33.4%) were from children attending Barau Dikko Specialist Hospital (BDSH), 125 (33.2%) from children attending Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and 126(33.4%) from children attending Institute for Child Health (ICH) Banzazzau Zaria.

One hundred and ninety-eight (198) representing 52.5% of the population studied were male and 179 (47.5%) were female, 107 (28.4%) of the children were within the age group 0 – 12 months and 105 (27.9%) were within the age group 13 – 24 months, 59 (15.4%), 52 (13.8%) and 54 (14.3%) were within the age groups 25 -35months, 37 -48 months and 49 -60 months respectively. One hundred and one 101(26.8%) of the children were in school (Day cares, Nursery and Primary) while 276 (73.2%) were not in school. Twenty (5.3%) of the children had orofacial lesions, 27(7.2%) had skin infections, 9(2.4%), 139 (36.9%),24 (6.4%) and158(41.9%) of the children had conjunctivitis, febrile illnesses, jaundice and other infections.

Analysis of Population by ELISA and IFA test

Two hundred and eighteen (218) of the 377 blood samples analysed by ELISA were seropositive to HSV-1 IgG antibody, representing a total prevalence of 57.8%, while 255 were positive by IFA test representing a prevalence of 67.6% (Table 1.0).In relation to the selected hospitals, the highest prevalence of 64% and 74.6% was recorded amongst children attending ABUTH and ICH by ELISA and IFA test respectively ,While children attending BDSH had the lowest prevalence of 49.2% and 58.7% by both ELISA and IFA test There was statistically significant association between HSV-1infection and the hospitals selected (x2 = 6.11, df=2, p=0.047).

The results were analysed according to age groups and children in age group 0-12months had the lowest prevalence of 57.9% by IFA while age group 0-12 and 13-24 months had the lowest prevalence by ELISA. Age group 49-60 months had the highest prevalence by both tests. Statistically significant association was found to exist between HSV-1infection and different age groups analysed.(x^2 = 37.92 df =4, p =0.001).In relation to the prevalence of the infection by gender, a higher prevalence of 61.5% by ELISA and 67.6% by IFA was observed in female children than in the male children (54.5% and 67.4%) by ELISA and IFA respectively. The differences observed in the prevalence by gender was not statistically significant(x^2 =1.84, df =1, P =0.105).

In relation to the educational status of children, a higher prevalence (74.3%) was observed in children who were in school than those who were not in school (51.8%). There was a statistically significant association between HSV-1infection and educational status of children by ELISA ($x2 = 15.28, df = 1, p = 0.001$) as shown in Table 1.0 and Children who were in school were more likely to be infected with HSV-1 infection than those who were not in school (OR=1.43, 95% CI = 0.5851 – 0.7898).However there was no association between HSV-1infection and educational status of children by IFA test (x2 = 1.99, df=1, p=0.98) though a higher prevalence was also recorded in children who were in school (94/101(73.3%)) than those who were not in school(181/276 (65.6%)).

The seroprevalence of HSV- 1 infection in relation to the clinical symptoms presented by the children showed the lowest prevalence in children with other infections such as sickle cell anaemia and respiratory tract infections while the highest prevalence was recorded in children with orofacial lesions (Table 1.0). Statistically significant association existed between the clinical symptoms presented by the children and seropositivity to HSV -1 infection(x^2 = 13.46, df =5, p=0.019).

Of the 377 blood samples analysed, 218 were seropositive to HSV -1 IgG antibody using ELISA test representing a seroprevalence of 57.8%, while 255 were positive by the IFA test with a seroprevalence of 67.6%.Ninety -nine of the blood samples analysed were negative by both tests, 23 and 60 were positive only by ELISA and IFA test respectively and 195 were seropositive by both tests (Table 2.0). Using ELISA as the goal standard in determining presence of HSV-1 infection, the sensitivity and specificity of the IFA test was determined to be 89.5% and 62.3% respectively.

Table 1: Percentage Seroprevalence of HSV-1 infection in children 0-5 years in Kaduna state, Nigeria.

Parameters	No. positive by *ELISA (%)	No. positive by **IFA (%)	Total (%)	p-value
Hospitals				
BDSH	62(49.2)	74(58.7)	126(33.4)	*0.047
ABUTH	80(64.0)	87(69.6)	125(33.2)	**0.023
ICH	76(60.3)	94(74.6)	126(33.4)	
Age(Months)				
0-12	48(44.9)	62(57.9)	107(28.4)	
13-24	47(44.8)	65(61.9)	105(27.9)	* 0.001
25-36	39(66.1)	43(72.8)	59(15.4)	**0.003
37-48	38(73.1)	39(75.0)	52(13.8)	
49-60	46(85.2)	48(88.9)	54(14.6)	
Gender				
Male	108 (54.5)	134(67.7)	198(52.5)	*0.105
Female	110 (61.5)	121(67.6)	179(47.5)	**1.00
Child's education				
In school	75(74.3)	94(73.3)	101(26.8)	*0.001
Not in school	143(51.8)	181(65.6)	276(73.2)	**0.98
Clinical symptoms				
Orofacial lesions	17(85.0)	15(75.0)	20(5.3)	
Skin infection	19(70.9)	16(59.3)	27(7.2)	
Conjunctivitis	7(77.8)	6(66.7)	9(2.4)	*0.019
Febrile illness	81(58.3)	101(72.7)	139(36.9)	**0.49
Jaundice	15(62.5)	17(70.8)	24(6.4)	
Others	79(50.0)	100(63.3)	158(41.9)	
Total	218(57.8)	255(67.6%	377(100)	

Figures in parentheses represent percentage

Key

BDSH-Barau Dikko Specialist Hospital

ABUTH-Ahmadu Bello University Teaching Hospital

ICH-Institute for Child Health

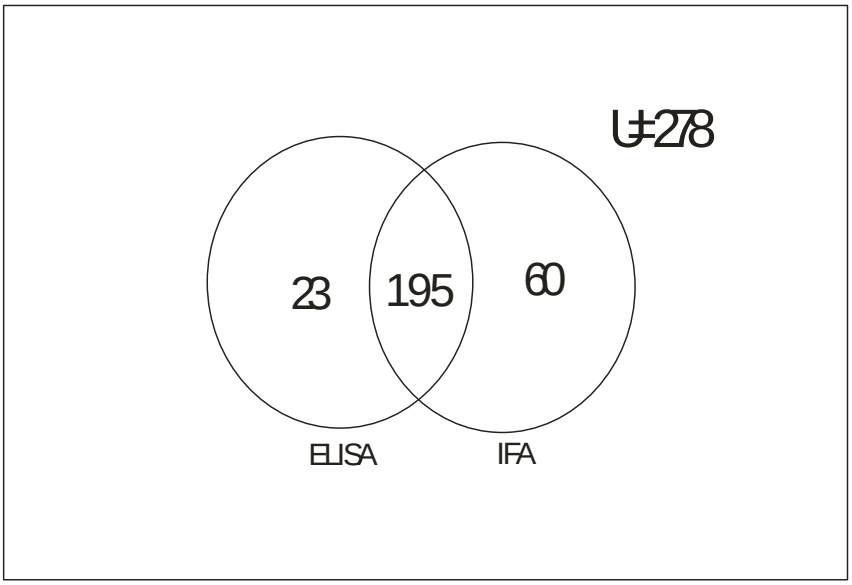

Figure 1: Relationship between ELISA and IFA Results

Table 2: Determination of sensitivity and specificity of IFA using the ELISA Test.

IFA	ELISA		Total
	Negative	Positive	
Negative	99	23	122
Positive	60	195	255
Total	**159**	**218**	**377**

$$Sensitivity = \frac{TruePositive}{TruePositive + FalseNegative} x\ 100$$

$$Sensitivity = \frac{195}{195 + 23} x\ 1oo$$

$$Sensitivity = \frac{195}{218} x\ 100$$

$$Sensitivity = 89.5\%$$

$$Specificity = \frac{TrueNegative}{TrueNegative + FalsePositive} x\ 100$$

$$Specificity = \frac{99}{99 + 60} x\ 1oo$$

$$Specificity = \frac{99}{159} x\ 100$$

$$Specificity = 62.3\%$$

DISCUSSION

This cross-sectional study evaluated the sero-prevalence of HSV-1 IgG antibody collected from 377 children within the ages of 0 and 5 years in some selected hospital in Kaduna state, Nigeria. Results obtained from this study showed a prevalence of 57.8% by ELISA and 67.6% by IFA test, The higher prevalence rate obtained by IFA test may be associated with some nonspecific fluorescence, which may not be as a result of the serum IgG against HSV -1 infection binding to the HSV -1 infected cells coated on the substrate slides and/or due to auto immune staining. This study is in agreement with sero-prevalence obtained in atopic children in Turkey which revealed a prevalence of 62.6% with ELISA and 57.3% with Virus Neutralization Test (VNT) (Fusun *et al.*, 2007).

Using ELISA test as the gold standard in determining HSV-1 infection (Fusun *et al.*, 2007), the sensitivity and specificity of the IFA test was calculated to be 89.5% and 62.3% respectively implying that ELISA still remains the best serological test for detection of HSV-1 infection (Cowan *et al.*,2003;Fusun *et al.*, 2007).

An overall seroprevalence of 57.8% was obtained from the study based on ELISA analysis and the results is similar to that obtained in children in Latin America and Caribbean (57.2%), Syria (55%) and Brazil (67.2%) (Ibrahim *et al.*, 2000; Costa Clemens and Farhat, 2010; Sukik *et al.*, 2019). The result is higher than those reported from Germany (31%), Israel (38%) and United States (47.8%) (Wutzler et al., 2000; Isacsohn *et al.*, 2002; McQuillan *et al.*, 2018).

The prevalence was lower than prevalences obtained in Nigeria such as 99.7% obtained among older children in Kaduna state (Abdulfatai, 2011) and 69% among adults in Plateau state (Rinmecit, 1985). The lower prevalence obtained in this study may be as a result of age difference in the study population. Earlier studies have found the prevalence of HSV-1 to increase with age progressively (Costa Clemens and Farhat, 2010; Vyse *et al.*,2000;Kasubi *et al.*,2006;Xu *et al.*,2007;Lin *et al.*,2011;McQuillan *et al.*, 2018).The published studies in Nigeria sampled older children and adults while the present studies sampled children 0-5 years old (younger children).

There was variation in the prevalence of HSV-1infection in the selected hospitals; the low prevalence seen in BDSH could be due to a number of factors. It could be due to higher cases of neonates and younger children seen in the unit who may not have had contact with the virus, the hospital is a primary/secondary health care facility and usually the first point of contact with patients and is situated in Kaduna which is an urban centre, may explain the low prevalence to be probably as a result of increased personal hygiene amongst members of the populace. The higher prevalence in ABUTH may be due to increased number of patients attending the hospital and probably due to the fact that ABUTH is a tertiary health care centre and so patients with clinical disease are more likely to be referred.

The higher prevalence obtained in females from the study agrees with the findings in Europe and USA where females are more likely to be HSV-1 seropositive than males (Vyse *et al.*,2000;Pebody *et al.*,2004; McQuillan *et al.*, 2018). The higher prevalence observed in female from this study may be by chance since the difference observed was not significant (P =0.105).

Older children had the highest prevalence of IgG antibody to HSV-1infection in this study; and antibodies to HSV-1 increased with increasing age. Detection of HSV- 1 infection with highest prevalence with increasing age agrees with the findings in Tanzania, Germany and Israel where HSV-1was detected with increasing prevalence with increasing age (Wutzler *et al.*, 2000; Isacsohn *et al.*, 2002;Kasubi *et al.*, 2006).

The prevalence obtained for children in age group 0-12 months and 13-24 months were similar. The prevalence of 44.8% in age group 0-12 months in this study is similar to the prevalence (49%) obtained in newborns in England and Wales (Vyse *et al.*, 2000). However, prevalence obtained in this age group could reflect in part maternal antibody (IgG) status, since the antibodies can cross the placenta. The decrease in prevalence (44.8%) in age group 13-24 months could be due to waning of maternal antibody. Studies have also revealed that early childhood stress can also elevate antibody levels in these children (Shirtchiff *et al.*, 2009). The highest prevalence of the infection in age group 49-60 months could be associated with increased level of interaction among children.

There was a statistically significant association in the prevalence of the infection with the educational status of the children. However, reported studies carried out did not compare the seropositivity to the infection and children educational status. The increased prevalence in children who attended formal education may be associated with the higher degree of interaction amongst children with their peers from variable cultures.

The clinical symptoms associated with HSV-1 infection in the study include: orofacial lesions, skin infection, conjunctivitis, jaundice, and febrile illnesses .This results is also in agreement with findings in Turkey (Fusun *et al.*, 2007). Most of the patients in the study presented with fever while a few of the children were suffering from sickle cell anaemia and respiratory tract infections. However, medical records were not obtained from the hospitals to know the relation of these conditions with increased frequency of HSV-1 infection.

Comparison between ELISA and IFA test in determining seroprevalence to HSV-1 infection indicated statistical difference between the two tests with a positive correlation of 0.56 and the measure of agreement between techniques was found to be 0.534 implying that the two assays agree by 53.4% which means there is only a partial agreement between these techniques, Therefore IFA test cannot be used in place of ELISA test in determining seroprevalence to HSV-1 infection.

CONCLUSION

The detection of HSV-1 IgG by ELISA provides a powerful and rapid method for investigation of HSV-1 past infection. Results obtained from this study indicated a prevalence of 57.8% by ELISA and 67.4% by IFA test, which is high and could in part be attributed to mode of transmission of the infection (direct contact with lesions and through saliva) implying that over 40% of the children are still susceptible to this infection and these pockets of unimmunized children are at risk of contracting the infection and subsequently coming down with the severe and devastating manifestation of the disease. From this study, HSV-1 childhood infection in Kaduna State was found to be associated with a number of factors such as age, children's educational status and clinical symptoms presented by the children.

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